

MRI of post mortem multiple sclerosis brain: quantifying the effects of formalin fixation

K. D. Schmierer¹, D. J. Tozer¹, C. A. Wheeler-Kingshott¹, P. A. Boulby¹, H. G. Parkes¹, T. A. Yousry¹, P. S. Tofts¹, D. H. Miller¹

¹NMR Research Unit, Institute of Neurology, UCL, London, England, United Kingdom

INTRODUCTION

Quantitative magnetic resonance imaging (qMRI) of *post mortem* (PM) multiple sclerosis (MS) brain is being used to probe MS (1,2). The acquisition and handling of fresh PM tissue are more challenging than of fixed tissue. However, limited data are available about the changes of qMRI indices in the brain following fixation (3-5). This study provides a framework for the interpretation of T₁ relaxation time (T₁), magnetisation transfer ratio (MTR), fraction of macromolecular protons (f_B), mean diffusivity (MD) and fractional anisotropy (FA) and their association with quantitative pathological indices in PM MS brain before and after formalin fixation.

METHODS

Fifteen fresh PM MS brain slices (mean age 58 years [SD 14], disease duration 25 years [11]) were studied 51 hours [28] PM, and again after 64 days [42] of formalin fixation. On a 1.5T GE Signa the following datasets were acquired (slice thickness=5mm): (i) spin echo (SE) T₁- and T₂-weighted (w) images, (ii) 2 sets of 2D GRE (TR 1500/36ms, TE 11ms, flip angle 45°) to calculate T₁, (iii) 2 sets of SE images to calculate MTR (TR 1720ms, TE 80ms, with/-out saturation prepulse), (iv) multi-shot diffusion-weighted SE-EPI (*b* =0, 1940s/mm²; TR=3s; TE=86 ms), (v) 2 D spoiled GRE (TR/TE 1140/12 ms) with 10 combinations of MT pulse amplitudes and offset frequencies from which f_B was calculated. After scanning tissue blocks were dissected and processed for embedding in paraffin. Sections were stained for Hematoxylin & Eosin, Luxol-Fast blue [LFB] and Bielschowsky's silver impregnation. Immunohistochemistry included glial fibrillary acidic protein (GFAP) and CD68. Lesions were classified as active, chronic active or chronic inactive and as demyelinated and remyelinated. Transmittance (Tr) was obtained from LFB- and GFAP-stained slides to assess myelin concentration (Tr_{myelin}) and severity of gliosis (Tr_{gliosis}), point-counting was used to quantify axons². T-tests of patient means (averaged across tissue samples) and linear regression were used.

RESULTS

Fifty-one lesions, identified on T₂w scans, and 15 regions of NAWM were included. Forty-four/51 lesions (86 %) were reproducible on T₂w MRI after formalin fixation. All qMR indices differed between lesions and NAWM pre- and post-fixation. Reduced values were detected after fixation for T₁ in lesions (1186ms pre- vs. 747ms post-fixation) and NAWM (668 vs. 377ms), for MD in lesions (357 vs. 309x10⁻⁶ mm²s⁻¹) and NAWM (222 vs. 161 x10⁻⁶ mm²s⁻¹), and in NAWM for MTR (33.9 vs. 30.0pu) and f_B (10.4 vs. 7.7 pu). No changes were detected for MTR and f_B in lesions, and for FA in lesions and NAWM. Strong correlation between qMR indices obtained pre- and post-fixation was detected (including both lesions and NAWM) for T₁ (*r*=0.77, *p*<0.001), f_B (*r*=0.89, *p*<0.001), MD (*r*=0.82, *p*<0.001), FA (*r*=0.77, *p*<0.001) and less so for MTR (*r*=0.63, *p*<0.001). In both conditions (fresh/fixed) all qMR indices were predictors of myelin content (tables 1,2). Multivariate regression revealed that in fresh tissue MTR was the strongest predictor of myelin. After fixation the strongest predictor of myelin was T₁ followed by f_B, whereas the association between myelin content and MTR dropped substantially. Axonal count and (to a much lesser extent) gliosis were also associated with qMR indices, however secondarily to their correlation with myelin (axons: *r*= 0.80, *p*<0.001; gliosis: *r*= 0.54, *p*=0.002).

CONCLUSION

All investigated qMR indices (except for FA) were affected by formalin fixation. qMR indices clearly differed between lesions and NAWM before and after fixation and – with the exception of MTR – showed strong correlation between values obtained pre- and post-fixation. However, the correlations between qMR indices and histology are being modified through fixation. Our data suggest that – with cautious reference to the described changes – valuable information about the likely *in vivo* changes due to MS may be inferred from qMR of fixed PM brain. T₁ and f_B appear to be the most reliable measures of myelin content after formalin fixation.

REFERENCES

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Table 1 Correlation between variables in fresh tissue

	T1	MTR	f _B	MD	FA
MTR	<i>r</i> = -0.79 <i>p</i> <0.001				
f _B	<i>r</i> = -0.83 <i>p</i> <0.001	<i>r</i> = 0.83 <i>p</i> <0.001			
MD	<i>r</i> = 0.78 <i>p</i> <0.001	<i>r</i> = -0.81 <i>p</i> <0.001	<i>r</i> = -0.75 <i>p</i> <0.001		
FA	<i>r</i> = -0.62 <i>p</i> = 0.001	<i>r</i> = 0.56 <i>p</i> = 0.003	<i>r</i> = 0.59 <i>p</i> = 0.007	<i>r</i> = -0.64 <i>p</i> <0.001	
Tr _{myelin}	<i>r</i> = 0.77 <i>p</i> <0.001	<i>r</i> = -0.83 <i>p</i> <0.001	<i>r</i> = -0.72 <i>p</i> <0.001	<i>r</i> = 0.73 <i>p</i> <0.001	<i>r</i> = -0.78 <i>p</i> <0.001
Axons	<i>r</i> = -0.63 <i>p</i> <0.001	<i>r</i> = 0.80 <i>p</i> <0.001	<i>r</i> = 0.67 <i>p</i> = 0.001	<i>r</i> = -0.52 <i>p</i> = 0.007	<i>r</i> = 0.58 <i>p</i> = 0.002

Table 2 Correlation between variables in fixed tissue

	T1	MTR	f _B	MD	FA
MTR	<i>r</i> = -0.54 <i>p</i> = 0.003				
f _B	<i>r</i> = -0.87 <i>p</i> <0.001	<i>r</i> = 0.69 <i>p</i> <0.001			
MD	<i>r</i> = 0.76 <i>p</i> <0.001	<i>r</i> = -0.63 <i>p</i> <0.001	<i>r</i> = -0.82 <i>p</i> <0.001		
FA	<i>r</i> = -0.78 <i>p</i> <0.001	<i>r</i> = 0.69 <i>p</i> <0.001	<i>r</i> = 0.80 <i>p</i> <0.001	<i>r</i> = -0.82 <i>p</i> <0.001	
Tr _{myelin}	<i>r</i> = 0.89 <i>p</i> <0.001	<i>r</i> = -0.68 <i>p</i> <0.001	<i>r</i> = -0.86 <i>p</i> <0.001	<i>r</i> = 0.78 <i>p</i> <0.001	<i>r</i> = -0.83 <i>p</i> <0.001
Axons	<i>r</i> = -0.78 <i>p</i> <0.001	<i>r</i> = 0.48 <i>p</i> = 0.007	<i>r</i> = 0.61 <i>p</i> =0.002	<i>r</i> = -0.53 <i>p</i> = 0.003	<i>r</i> = 0.64 <i>p</i> <0.001